

# Glycogen Phosphorylase b (GPb) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Ultraviolet spectrophotometer

**Catalog Number:** BC3350

**Size:** 50T/24S

## Components:

Reagent	Size	Storage
Extract solution	30 mL×1	4°C
Reagent I	50 mL×1	4°C
Reagent II	Powder×1	4°C
Reagent III	Powder×1	4°C
Reagent IV	Powder×1	-20°C
Reagent V	Powder×3	-20°C
Reagent VI	Powder×3	-20°C
Reagent VII	Powder×1	4°C

Solution preparation:

- Reagent II:** Dissolved with 1.25 mL of distilled water before use. Mix thoroughly. It can be stored for 8 weeks at 4°C.
- Reagent III:** Dissolved with 1.25 mL of distilled water before use. Mix thoroughly. It can be divided into small tubules and stored for 4 weeks at -20°C. Avoid repeating freeze/thaw cycles.
- Reagent IV:** Dissolved with 1.25 mL of distilled water before use. Mix thoroughly. It can be divided into small tubules and stored for 4 weeks at -20°C. Avoid repeating freeze/thaw cycles.
- Reagent V:** Dissolved with 1 mL of distilled water before use. Mix thoroughly. It can be divided into small tubules and stored for 4 weeks at -20°C. Avoid repeating freeze/thaw cycles.
- Reagent VI:** Dissolved with 1 mL of distilled water before use. Mix thoroughly. It can be divided into small tubules and stored for 4 weeks at -20°C. Avoid repeating freeze/thaw cycles.
- Reagent VII:** Dissolved with 4 mL of distilled water before use. Mix thoroughly. It can be divided into small tubules and stored for 4 weeks at -20°C. Avoid repeating freeze/thaw cycles.
- Working solution:** Calculate according to the amount required for the experiment before use, according to the ratio of reagent I: reagent II: reagent III: reagent IV= 740μL: 20μL: 20μL: 20μL(amount of 1T), mix well before use.

## Product Description:

Glycogen phosphorylase is divided into active glycogen phosphorylase a (Glycogen phosphorylase a, GPa) and inactive glycogen phosphorylase b (Glycogen phosphorylase b, GPb) two forms. The decomposition of glycogen is mainly carried out under the catalysis of glycogen phosphorylase a. When no

activator is added, glycogen phosphorylase a catalyzes the production of glucose residues from glycogen and inorganic phosphorus to glycogen and glucose 1-phosphate. Under the action of phosphoglucose mutase and 6-phosphate glucose dehydrogenase, it further catalyzes the reduction of NADP to NADPH. Measuring the rate of increase of NADPH at 340nm can reflect the activity of glycogen phosphorylase a. The activity of GP (GP<sub>a</sub> and GP<sub>b</sub>) is measured by adding an activator, and GP<sub>a</sub> activity is measured when no activator is added. GP<sub>b</sub> activity can be obtained by subtracting GP<sub>a</sub> activity from GP activity.

### Reagents and Equipment Required but Not Provided:

Ultraviolet spectrophotometer, low temperature centrifuge, constant temperature incubator /water bath, adjustable pipette, mortar/homogenizer, 1 mL quartz cuvette, ice and distilled water.

### Procedure

#### I. Sample preparation:

1. Tissue sample: according to the proportion of tissue weight (g): extraction solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extraction reagent and fully homogenized on ice bath. Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice before testing.
2. Bacteria or cells: collecting bacteria or cells into the centrifuge tube, suggested 5 million with 1 mL of Extraction reagent. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 200w, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials, and take the supernatant on ice for testing.
3. Serum (plasma) sample: detect sample directly.

#### II. Determination procedure:

1. Preheat ultraviolet spectrophotometer for 30 minutes, adjust wavelength to 340 nm, set zero with distilled water.
2. Working solution are preheated at 37°C for 5min.
3. GP<sub>a</sub> activity: add 50 μL sample, 50 μL reagent V, 50 μL reagent VI, 50 μL distilled water, 800 μL working solution in the 1mL quartz cuvette, mix immediately, and record the absorbance value at 10s at 340 nm A<sub>1</sub>, quickly place it in 37°C water bath or incubator for 10 minutes, take it out and quickly dry it and measure the absorbance value A<sub>2</sub> in 10min10s. Calculate  $\Delta AGPa = A_2 - A_1$ .
4. GP activity: add 50 μL sample, 50 μL reagent V, 50 μL reagent VI, 50 μL reagent VII, 800 μL working solution in the 1mL quartz cuvette, mix immediately, and record the absorbance value at 10s at 340 nm A<sub>1</sub>, quickly place it in 37°C water bath or incubator for 10 minutes, take it out and quickly dry it and measure the absorbance value A<sub>2</sub> in 10min10s. Calculate  $\Delta AGP = A_2 - A_1$ .

#### III. Calculations:

##### A. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol

NADPH per minute every milligram protein in 37°C.

$$GP_b \text{ (U/mg prot)} = [(\Delta A_{GP} - \Delta A_{GPa}) \times V_R \div (\epsilon \times d) \times 10^9] \div (V_S \times C_{pr}) \div T = 321.54 \times (\Delta A_{GP} - \Delta A_{GPa}) \div C_{pr}$$

#### B. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every gram tissue in 37°C.

$$GP_b \text{ (U/g weight)} = [(\Delta A_{GP} - \Delta A_{GPa}) \times V_R \div (\epsilon \times d) \times 10^9] \div (W \times V_S \div V_E) \div T = 321.54 \times (\Delta A_{GP} - \Delta A_{GPa}) \div W$$

#### C. Sample volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every milliliter volume in 37°C.

$$GP_b \text{ (U/mL)} = [(\Delta A_{GP} - \Delta A_{GPa}) \times V_R \div (\epsilon \times d) \times 10^9] \div V_S \div T = 321.54 \times (\Delta A_{GP} - \Delta A_{GPa})$$

#### D. Bacteria or cells number

Unit definition: One unit of enzyme activity is defined as the amount of enzyme produce 1 nmol NADPH per minute every million bacteria or cells in 37°C.

$$GP_b \text{ (U/10}^4 \text{ cells)} = [(\Delta A_{GP} - \Delta A_{GPa}) \times V_R \div (\epsilon \times d) \times 10^9] \div (\text{cell number} \times V_S \div V_E) \div T = 321.54 \times (\Delta A_{GP} - \Delta A_{GPa}) \div \text{cell number}$$

$\epsilon$ : NADPH molar extinction coefficient, 6220 L/mol/cm;

d: Cuvette light path, 1cm;

$V_R$ : Total reaction volume, 1 mL;

$V_S$ : Add sample volume, 0.05mL;

$V_E$ : extract volume, 1 mL;

W: Sample weight, g;

C<sub>pr</sub>: Protein concentration of sample, mg/mL;

T: Reaction time, 10min

#### Note:

- If the measured absorbance value  $\Delta A > 0.6$ , it is recommended to dilute the sample before measuring, and multiply the dilution factor in the calculation formula; if the measured absorbance value is low or close to the blank OD value, it is recommended to increase the sample volume before performing the measurement

#### Experimental example

- Take 0.1 g of rabbit liver tissue, add 1 mL of extract, homogenize in an ice bath, centrifuge at 8000 ×g for 10 minutes at 4°C; take the supernatant and place on ice for testing. Use 1mL quartz cuvette to operate according to the determination steps, calculate

$\Delta A_{GPa} = A_2 - A_1 = 0.414 - 0.318 = 0.096$ ,  $\Delta A_{GP} = A_4 - A_3 = 0.434 - 0.320 = 0.114$ , according to the formula

Calculated activity:

$$GPb \text{ (U/g weight)} = 321.54 \times (\Delta A_{GP} - \Delta A_{GPa}) \div W = 57.87 \text{ U/g weight}$$